Intersection between ageing-related Genes and SARS-CoV-2 Interactome: Is it higher than expected?

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Method Article

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (coronavirus disease, 2019; COVID-19) is associated with adverse outcomes in patients. It has been observed that lethality seems to be related to the age of patients. Moreover, it has been demonstrated that ageing causes some modifications at a molecular level.

Objective: The study aims to investigate a possible link between the increased COVID-19 lethality and the molecular changes that occur in elderly people.

Methods: We considered publicly available datasets on ageing-related genes and SARS-CoV-2 interactors. Then, for each SARS-CoV-2 protein interactor, we tested for the enrichment of ageing-related proteins. Finally, we performed a network-based analysis to identify which molecular mechanisms could play a role in the SARS-CoV-2 molecular aetiology and ultimately affect COVID-19 outcome.

Results: We observed a significant intersection between some SARS-CoV-2 interactors and ageing-related genes. Our analysis evidenced that virus infection particularly affects ageing molecular mechanisms centred around proteins *EEF2*, *NPM1*, *HMGA1*, *HMGA2*, *APEX1*, *CHEK1*, *PRKDC*, and *GPX4*.

Conclusion: Our study generated a mechanistic framework aiming at explaining the correlation between COVID-19 incidence in elderly patients and molecular mechanisms of ageing. This will provide testable hypotheses for future investigation on the mechanism of action of coronaviruses and pharmacological solutions tailored on specific age ranges.

1. Introduction

At the end of 2019 in Wuhan (China), medical facilities reported acute pneumonia cases with an unknown origin. Further analysis revealed that a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was responsible for that disease, called coronavirus disease 2019 (COVID-19) [1]. From the beginning, the clinical manifestations spanned from asymptomatic infection to severe pneumonia as well as a severe inflammation state (molecularly characterised by a cytokine cascade [2]) with a fatal outcome [3, 4, 5, 6].

Starting from China, the virus spread in almost all other countries globally, causing infections and deaths. On 11th March 2020, the World Health Organisation (WHO) declared SARS-CoV-2 as a pandemic. Current data [7] revealed that the impact of COVID-19 presents certain peculiar aspects in different nations that have been deeply investigated. Some authors hypothesised that virus mutations were responsible for these differences [8, 9, 10]. Nevertheless, many independent studies agreed that the mutations did not have a primary role in the differentiation of the outcomes [11, 12, 13].

Despite the lack of the individuation of the causes, there was a substantial agreement on the fact that the variation of the case fatality rate (CFR), i.e. the fraction of confirmed case with lethal outcome, ranging from 0 to 20% and beyond at country level, should be deeply investigated [14, 15, 16]. Among the other

differences, we focus on the observation that the infection is significantly more lethal in elderly people [17, 18, 19, 20]. Some studies have focused on the possible link between increased mortality rate and some characteristics of elderly people [21, 22]. These studies suggested the potential effect of the virus as a trigger activating the decompensation of other chronic conditions [23, 24, 25, 26]. In [27] authors discuss the increased state of inflammation in old people, (called inflammageing), and its possible relation with COVID-19 manifestation that causes the rise of inflammation.

Previous studies [28] have also evidenced that the understanding of modification of molecular mechanism related to the ageing process (i.e. modification of gene expression, modulation of regulatory mechanisms) may reveal important insight about ageing. Many studies contributed to identifying such ageing-related diseases [29, 30, 31] despite the difficulty of having experimental data [32]. However, wet lab experimental analyses of human ageing are difficult to find. Computational predictions have also been made in [28, 33] giving both candidate genes and networks [34, 35].

Consequently, the study of the intersection between SARS-CoV-2 and ageing-related molecular alterations could augment the understanding of COVID-19 and then improve its treatment [36]. In [37] a first analysis based on some preliminary public data has reinforced the rationale that there is a possible link.

We start from the consideration that a possible link exists; we hypothesise that SARS-CoV-2 interacting proteins (and genes) may have an overlap with ageing-related genes higher than chance. Therefore, the infection may dysregulate these mechanisms that are yet altered in elderly people causing severe outcomes. We explored such scenario using available public data of expression changes in elderly people GTEx data [38], genes annotated as ageing genes in MSigDB [39], and interacting partners of SARS-CoV-2 described in [40, 41].

We observed a significant overlap between the interactors of SARS-CoV-2 and ageing group. Further, we looked at a network-level scenario [35], by considering possible regulatory mechanisms that may be altered [42, 43]. These observations support previous reports that SARS-CoV-2 may manifest in the blood leading to multiorgan failure in severe cases of COVID-19.

2. Methods

SARS-CoV-2 Interaction Map We considered SARS-CoV-2 protein interaction map provided by Gordon et al., [41] and by Guzzi et al., [40]. Both works provided data about 26 of the 29 SARS-CoV-2 proteins behaviour in human cells by identifying the human proteins that physically associated with each of the SARS-CoV-2 proteins using affinity-purification mass spectrometry. They found high-confidence protein-protein interactions between SARS-CoV-2 and human proteins; they also provided data about possible interactions with an associated reliability score. We considered both high and low confidence interactions.

Ageing Related Genes We first defined and labelled genes (and or proteins) as ageing. We considered data provided from GTEx dataset that contains genes positively and negatively correlated with human age [38]. We gathered data from the GenAge dataset that derived human genes by projecting sequence orthologs in model organisms. We also considered MSigDB, which collected 70 studies associated with ageing. We

excluded from the analysis datasets containing data from the brain or other tissues that are not related to COVID-19 manifestation, thus focusing only on lung and kidney data. We only selected datasets reporting experiments from homo sapiens since the projection of orthologs may produce not reliable results for ageing as described in [28].

Bioinformatic Analysis We considered SARS-CoV-2 interacting partners. We measured the intersection between its list of interactors and the ageing-related genes for each viral protein. We estimated the probability that this intersection is higher than chance.

Database of Protein Interactions We used the Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING) [44] that is a freely available repository storing both physical and functional association among proteins. Users may search the database through a web interface by specifying a protein identifier or inserting the primary sequence of a protein. We queried the database using the identifiers of the nodes of each subnetwork. We used medium confidence as the minimum confidence score for each interaction and all for the sources of interactions.

Network Analysis For each subnetwork, we report the main topological parameters: number of nodes, number of edges, average node degree, average local clustering coefficient, the expected number of edges, and PPI enrichment p-value. A p-value lower than 0.01 indicates that the input list of proteins has more interactions than what would be expected for a random set of proteins of similar size, drawn from the genome. For each network we performed a Gene Ontology enrichment analysis, and we take into account only top enriched annotation considering the strength measure. This measure is the log-ratio between i) the number of proteins in each network that are annotated with a term and ii) the number of proteins that we expect to be annotated with this term in a random network of the same size. For each network, we also extracted the main pathways associated in the Reactome database [45]. Network analysis and visualisation were performed in Cytoscape 3.7.0 [46].

3. Results

We report the results of the significance of intersection of virus interactors and ageing related genes. Table 1 reports the p-values of the intersection. We set as threshold a p value 0:01.

SARS-CoV2 M The analysis revealed that only ten viral proteins have interactors with a significant overlap with respect to ageing related proteins as summarised in Table 1. For each interactor we report human proteins. Figure 1 reports all the subnetworks of SARS-CoV-2 interactors related to ageing.

3.1 Interaction networks of the Interactors.

M Subnetwork Figure 2 reports the interactors of the M viral proteins. The subnetwork has 35 nodes and 61 edges. The STRING database reports a PPI enrichment p-value equal to $1.43e-13$ as reported in Table 2, therefore there is a significant probability that these interactors may be involved into a set of common functions. Main biological processes are senescence-associated heterochromatin focus assembly

(GO:0035986), oncogene-induced cell senescence (GO:0090402), and positive regulation of cell ageing (GO:0090403).

NSP2 Subnetwork The interaction network of NSP2 protein has 25 proteins and 45 edges. It has a relatively high node degree as reported in Table 3 and depicted in Figure 3. Main enriched biological processes (considering Gene Ontology Biological Processes) are: senescence-associated heterochromatin focus assembly (GO:0035986), positive regulation of cell ageing (GO:0090343). Main associated pathways in Reactome Database are: Deposition of new CENPA (HSA-606279) and Immune System (HSA-168256).

NSP4 Subnetwork The human ppi subnetwork induced by NSP4 interactors has 32 nodes and 45 edges as reported in Table 4 and depicted in Figure 4. Main enriched biological processes (considering Gene Ontology Biological Processes) are: senescence-associated heterochromatin focus assembly (GO:0035986), positive regulation of cell ageing (GO:0090343). Main associated pathways in Reactome Database are: Deposition of new CENPA (HSA-606279) and Immune System (HSA-168256).

NSP6 Subnetwork The human ppi subnetwork induced by NSP6 interactors has 28 nodes and 40 edges as reported in Table 5 and depicted in Figure 5. Main biological processes enriched in the network are: senescence-associated heterochromatin focus assembly (GO:0035986), oncogene-induced cell senescence (GO:0090402). Main pathways associated into the Reactome database are: Formation of Senescence-Associated Heterochromatin (HSA-2559584) and CDS1 mediated inactivation of Cyclins (HSA-75035).

NSP8 Subnetwork The human ppi subnetwork induced by NSP8 interactors has 18 nodes and 30 edges as reported in Table 6 and depicted in Figure 6. Main biological processes enriched are: senescence-associated heterochromatin focus assembly (GO:0035986), regulation of cellular senescence (GO:2000772), and positive regulation of cellular senescence (GO:2000774). Similarly, even the Reactome Pathways associated with this network are related to senescence and DNA: Formation of Senescence-Associated Heterochromatin (HSA-2559584), and DNA Damage/Telomere Stress Induced Senescence (HSA-2559586).

NSP11 Subnetwork The network associated to NSP11 interactors has 28 nodes and 44 edges as depicted in Figure 7 and as summarised in Table 7. Main biological processes associated to this network are: positive regulation of cellular senescence (GO:2000774), negative regulation of B cell apoptotic process (GO:0002903), and senescence-associated heterochromatin focus assembly (GO:0035986). Pathways associated are: Formation of Senescence-Associated Heterochromatin (HSA-2559584), Activation of E2F1 target genes at G1/S.

NSP13 Subnetwork The network associated to NSP13 interactors has 27 nodes and 58 edges as depicted in Figure 8 and as summarised in Table 8. Main biological processes associated to this network are: positive regulation of cellular senescence (GO:2000774), negative regulation of B cell apoptotic process (GO:0002903), and senescence-associated heterochromatin focus assembly (GO:0035986). Pathways associated are: Formation of Senescence-Associated Heterochromatin (HSA-2559584), Activation of E2F1 target genes at G1/S and Cds1 mediated inactivation of Cyclin B (HSA-75035).

Orf3a Subnetwork The network associated to Orf3a interactors has 33 nodes and 87 edges as depicted in Figure 9 and as summarised in Table 9. Main biological processes associated to this network are: positive regulation of cellular senescence (GO:2000774), negative regulation of B cell apoptotic process (GO:0002903), and senescence-associated heterochromatin focus assembly (GO:0035986). Pathways associated are Formation of Senescence-Associated Heterochromatin (HSA-2559584), Activation of E2F1 target genes at G1/S and Cds1 mediated inactivation of Cyclin B (HSA-75035) .

Orf8 Subnetwork The network associated to Orf8 interactors has 29 nodes and 40 edges as depicted in Figure 10 and as summarised in Table 10. Main biological processes associated to this network are: positive regulation of cellular senescence (GO:2000774), negative regulation of B cell apoptotic process (GO:0002903), and senescence-associated heterochromatin focus assembly (GO:0035986). Major pathways associated to this subnetwork are: Assembly of Viral Componentes at the Budding Site (HSA-168316), Formation of Senescence-Associated Heterochromatin (HSA-2559584), Activation of E2F1 target genes at G1/S and Cds1 mediated inactivation of Cyclin B(HSA-75035) .

Orf9C Subnetwork The network associated to Orf9c interactors has 42 nodes and 80 edges as depicted in Figure 11 and as reported in Table 11. Main biological processes associated to this network are: senescence-associated heterochromatin focus assembly (GO:0035986), positive regulation of cellular senescence (GO:2000774), ectopic germ cell programmed cell death (GO:0035234). Major pathways associated are: Formation of Senescence-Associated Heterochromatin (HSA-2559584), CDS mediated inactivation of Cyclin B (HSA-75035).

Common Subnetwork among the interactors subnetworks The Gene Ontology analysis reveals that the whole network is enriched with the following terms: (GO:0090402) oncogene-induced cell senescence, (GO:0035986) senescence-associated heterochromatin focus assembly, (GO:2000774) positive regulation of cellular senescence, (GO:2000773) negative regulation of cellular senescence, (GO:2000772) regulation of cellular senescence. The analysis of Reactome DB reveals that the subnetwork is associated with following pathways: Formation of Senescence Associated Heterochromatins Foci (HSA2559584), Host interactions of HIV factors.

4. Discussion

As investigated in [47] ageing is characterised by the decline of the immune function. Elderly people are not immuno-deficient, but often the response of their immune system is not sufficient to be effective again antigens. This effect is particularly evident when they are subject to novel antigens. It is known that both responses to influenza and vaccination are not efficient in elderly [48, 49]. Moreover, elderly accumulate inflammatory mediators in tissues (inflammageing process), that may occur by the accumulation of DNA lesions that in turn, trigger the increased production of inflammatory mediators [50]. In parallel, the link between COVID-19 and the suppression of the immune system has been observed in [51]. Authors found that many proteins related to the immune response were modulated, causing the possible suppression of such a system.

In this work we observed that some of the genes that are involved in this process (both inflammaging and immune response decline) [52] are targeted by the SARS-CoV-2, thus giving a partial answer to the increased mortality in elderly people. Interestingly, we found that the intersection between targets of SARS-CoV-2 and ageing-related proteins is higher than by chance for some virus proteins (NSP2, NSP4, NSP6, NSP11, NSP13, Orf7a, Orf3a, Orf8, Orf9c and M). This may suggest that virus may cause a different response in elderly people; thus, further experiments may be done to investigate this relation in orthogonal studies. We considered first interactors of each viral protein separately, then we considered interactions between the interactors and finally, we considered the common sub-networks of them.

First of all, it should be noted that the proteins have more interactions among themselves with respect to a random null model as reported by STRING database. It indicates that all these proteins may be biologically connected, i.e. they may belong to some correlated function or pathway. The Gene Ontology analysis revealed that the whole network is enriched with the following terms: (GO:0090402) oncogene-induced cell senescence, (GO:0035986) senescence-associated heterochromatin focus assembly, (GO:2000774) positive regulation of cellular senescence, (GO:2000773) negative regulation of cellular senescence, (GO:2000772) regulation of cellular senescence.

Moreover, the common sub-network reveals some insights in molecular mechanism related to senescence. The bioinformatics analysis reveals that the subnetwork is associated with previous infectious disease (HIV, SARS-CoV, influenza viruses) [53].

The High Mobility Group A (HMGA) chromatin architectural factors [54] have been associated in the past with the control of a number of pathways involved in cell proliferation and survival. The High Mobility Group A (HMGA) family includes three proteins: HMGA1a, HMGA1b, (produced by alternative splicing of the same gene) and HMGA2. These proteins alter chromatin structure and facilitate the assembly of transcriptional factors [55]. Therefore, they modulate the transcription of several genes involved in a wide spectrum of biological processes, such as embryogenesis, cell differentiation, cell cycle, cell migration, apoptosis and cell transformation. In particular, there is evidence of global changes of chromatin domain from in vitro studies for ageing [56]. In particular, it has been observed that during senescence mammalian cells develop senescence-associated heterochromatin foci as well as high-mobility group A (HMGA) proteins. Therefore, the dysregulation may accelerate cell cycle arrest in response to stress [57].

The elongation factor protein EEF2 promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome. It has been associated in the past with the [58] ageing processes related to the modulation of protein synthesis. In particular, the levels of EEF2 protein were related to longevity. Therefore, the dysregulation may cause the accumulation on DNA damage or on the protein levels accumulating errors in the downstream cascade of mechanisms [59]. The role of EEF2 in severe cases of COVID-19 has also been elucidated in [51]. Thus our study provides another evidence. Moreover, this protein is targeted together with the Eukaryotic translation initiation factor 2 subunit 1 (EIF2S1) by Orf3a, Orf8, NSP2, NSP6, NSP11, NSP13, indicating a possible role of the virus to promote viral translation over cellular translation [60].

The Nucleophosmin (NPM1) is involved in many cellular processes already related to DNA and cell cycle control such as ribosome biogenesis, protein chaperoning, centrosome duplication, histone assembly, and cell proliferation [61, 62]. Previous studies investigated the age incidence of acute myeloid leukaemia with mutated nucleophosmin (NPM1) [63, 64], while there are no studies related to these mutations and other diseases. Therefore, this work may suggest further studies in such a direction. The interaction between NPM1 and the nucleocapsid protein of the previous SARS-CoV is known to affect the viral particle assembly [65, 66, 67]. The role of NPM1 and of Histone H2AX targeted by other viral proteins has also been reported in other viruses such as Epstein-Barr and KSHV as a common strategy to manipulate translation and to promote virus latency [68, 69].

The DNA (apurinic or apyrimidinic site) lyase (APEX1) plays an important role in the cellular response to oxidative stress. APEX1 has a major role in DNA-repair and in redox regulation of transcription factors [64]. It positively acts in DNA base excision repair (BER) pathway of DNA lesions induced by oxidative and alkylating agents. Together with NPM1, associates with rRNA and Binds DNA and RNA. The modifications in the APEX expression have been correlated with increased senescence [64]. In [70] the synergistic downregulation of both APEX and NPM1 has been clearly observed in oligodendrocyte cells in relation to ageing.

The DNA-dependent protein kinase catalytic subunit (PRKCD) is a Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. It also plays a role in the regulation of DNA virus-mediated innate immune response by assembling into the HDP-RNP complex [71]. This finding is compatible with the consideration that COVID-19 pathogenesis is also related with inflammation-mediated coagulation as a result of inflammatory responses as reported [72].

Also, the CHEK1 is a serine/threonine-protein kinase which is required for checkpoint-mediated cell cycle arrest and activation of DNA repair in response to the presence of DNA damage or unreplicated DNA. It participates in the regulation of a set of mechanisms related to the preservation of the integrity of the genome [73]. The involvement of this protein suggests the severe inhibition of the DNA damage response system during virus infection leading to acute inflammation. Moreover, CHEK1 is targeted together with CDK1 by many SARS-CoV-2 interactors (NSP2, NSP4, NSP11, NSP13) and with CDKN2A (Orf3, NSP13) suggesting an additive effect on the disruption of pathways of apoptosis mediated by TP53 [74] yet dysregulated by both senescence and ageing. [75].

5. Conclusion

We applied a bioinformatic analysis to perform a qualitative analysis of mechanisms of infection by SARS-CoV-2 in elderly people.

Several studies have shown in the past the modifications of genes and proteins that occur in elderly people. Other studies have partially elucidated the mechanism of infections and the dysregulated pathways in COVID patients.

We detected a statistically significant overlap between SARS-CoV-2 interacting proteins and those related to ageing, suggesting a potentially different response in elderly people. These results will provide an important molecular basis for understanding the mechanism of infections and will shed light on infection progression.

The limitation of this study is that the dataset is correlative, and thus it should be confirmed by in-vivo experiments.

Declarations

Conflict of Interests

Authors state that they do not have conflict of interest.

Author Contribution

F.M.G and P.H.G. conceived the main idea of this manuscript. Both participated to the experimental phase and on the discussion of the results. E.P. participated to writing of Discussion Section. All authors read and approved the manuscript.

References

- [1] Talha Burki. Outbreak of coronavirus disease 2019. *The Lancet Infectious Diseases*, 20(3):292–293, 2020.
- [2] Gholam Hossein Meftahi, Zohreh Jangravi, Hedayat Sahraei, and Zahra Bahari. The possible pathophysiology mechanism of cytokine storm in elderly adults with covid-19 infection: the contribution of “inflame-aging”. *Inflammation Research*, pages 1–15, 2020.
- [3] Chaolin Huang, Yeming Wang, Xingwang Li, Lili Ren, Jianping Zhao, Yi Hu, Li Zhang, Guohui Fan, Jiuyang Xu, Xiaoying Gu, et al. Clinical features of patients infected with 2019 novel coronavirus in wuhan, china. *The Lancet*, 395(10223):497–506, 2020.
- [4] Dawei Wang, Bo Hu, Chang Hu, Fangfang Zhu, Xing Liu, Jing Zhang, Binbin Wang, Hui Xiang, Zhenshun Cheng, Yong Xiong, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in wuhan, china. *Jama*, 323(11):1061–1069, 2020.
- [5] Christopher M Petrilli, Simon A Jones, Jie Yang, Harish Rajagopalan, Luke O’Donnell, Yelena Chernyak, Katie A Tobin, Robert J Cerfolio, Fritz Francois, and Leora I Horwitz. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in new york city: prospective cohort study. *bmj*, 369, 2020.

- [6] Flavia Biamonte, Cirino Botta, Maria Mazzitelli, Salvatore Rotundo, Enrico Maria Trecarichi, Daniela Foti, Carlo Torti, Giuseppe Viglietto, Daniele Torella, and Francesco Costanzo. Combined lymphocyte/monocyte count, d-dimer and iron status predict covid-19 course and outcome in a long-term care facility. Researchsquare, 2020.
- [7] Ensheng Dong, Hongru Du, and Lauren Gardner. An interactive web-based dashboard to track covid-19 in real time. *The Lancet infectious diseases*, 20(5):533–534, 2020.
- [8] Juan David Ramírez, Marina Muñoz, Carolina Hernández, Carolina Flórez, Sergio Gomez, Angelica Rico, Liseth Pardo, Esther C Barros, and Alberto E Paniz-Mondolfi. Genetic diversity among sars-cov2 strains in south america may impact performance of molecular detection. *Pathogens*, 9(7):580, 2020.
- [9] Bette Korber, Will M Fischer, Sandrasegaram Gnanakaran, Hyejin Yoon, James Theiler, Werner Abfalterer, Nick Hengartner, Elena E Giorgi, Tanmoy Bhattacharya, Brian Foley, et al. Tracking changes in sars-cov-2 spike: evidence that d614g increases infectivity of the covid-19 virus. *Cell*, 182(4):812–827, 2020.
- [10] Ida Gagliardi, Gemma Patella, Ashour Michael, Raffaele Serra, Michele Provenzano, and Michele Andreucci. Covid-19 and the kidney: From epidemiology to clinical practice. *Journal of Clinical Medicine*, 9(8):2506, 2020.
- [11] Daniele Mercatelli, Andrew N Holding, and Federico M Giorgi. Web tools to fight pandemics: the covid-19 experience. *Briefings in Bioinformatics*, 2020.
- [12] Francesco Ortuso, Daniele Mercatelli, Pietro Hiram Guzzi, and Federico Manuel Giorgi. Structural genetics of circulating variants affecting the sars cov-2 spike/human ace2 complex. *bioRxiv*, 2020.
- [13] Johnah C Galicia, Pietro H Guzzi, Federico M Giorgi, and Asma A Khan. Predicting the response of the dental pulp to sars-cov2 infection: a transcriptome-wide effect cross-analysis. *Genes & Immunity*, 21(5):360–363, 2020.
- [14] Gabriele Sorci, Bruno Faivre, and Serge Morand. Explaining among-country variation in covid-19 case fatality rate. *Scientific reports*, 10(1):1–11, 2020.
- [15] Wayne C Koff and Michelle A Williams. Covid-19 and immunity in aging populations—a new research agenda. *New England Journal of Medicine*, 2020.
- [16] Gabriella Marcon, Mauro Tettamanti, Giorgia Capacci, Giulia Fontanel, Marco Spanò, Alessandro Nobili, Gianluigi Forloni, and Claudio Franceschi. Covid-19 mortality in lombardy: the vulnerability of the oldest old and the resilience of male centenarians. *Aging (Albany NY)*, 12(15):15186, 2020.
- [17] Smriti Mallapaty. The coronavirus is most deadly if you are older and male—new data reveal the risks. *Nature*, pages 16–17, 2020.
- [18] Tao Li, Lei Lu, Weishuo Zhang, Yu Tao, Liuming Wang, Jing Bao, Bao Liu, and Jun Duan. Clinical characteristics of 312 hospitalized older patients with covid-19 in wuhan, china. *Archives of gerontology*

and geriatrics, 91:104185, 2020.

[19] Carlo Vittorio Cannistraci and Ilaria Capua. Age-adaptive and gender-balanced social interventions might be stepping stones to the next 'new normal' in covid-19 outbreak. Preprints, 2020.

[20] Seung-Ji Kang and Sook In Jung. Age-related morbidity and mortality among patients with covid-19. *Infection & chemotherapy*, 52(2):154, 2020.

[21] Anna Julia Pietrobon, Franciane Mouradian Emidio Teixeira, and Maria Notomi Sato. Immunosenescence and inflammaging: Risk factors of severe covid-19 in older people. *Frontiers in Immunology*, 11, 2020.

[22] Jon Hazeldine and Janet M Lord. Immunesenescence: a predisposing risk factor for the development of covid-19? *Frontiers in immunology*, 11:2381, 2020.

[23] Claudio Napoli, Isabella Tritto, Gelsomina Mansueto, Enrico Coscioni, and Giuseppe Ambrosio. Immunosenescence exacerbates the covid-19. *Archives of Gerontology and Geriatrics*, 2020.

[24] Giacomo Grasselli, Antonio Pesenti, and Maurizio Cecconi. Critical care utilization for the covid-19 outbreak in lombardy, italy: early experience and forecast during an emergency response. *Jama*, 323(16):1545–1546, 2020.

[25] Annemarie B Docherty, Ewen M Harrison, Christopher A Green, Hayley E Hardwick, Riinu Pius, Lisa Norman, Karl A Holden, Jonathan M Read, Frank Dondelinger, Gail Carson, et al. Features of 20 133 uk patients in hospital with covid-19 using the isaric who clinical characterisation protocol: prospective observational cohort study. *bmj*, 369, 2020.

[26] Eleonora Cianflone, Michele Torella, Flavia Biamonte, Antonella De Angelis, Konrad Urbanek, Francesco S Costanzo, Marcello Rota, Georgina Mellison-Hughes, and Daniele Torella. Targeting cardiac stem cell senescence to treat cardiac aging and disease. *Cells*, 9(6):1558, 2020.

[27] Arne N. Akbar and Derek W. Gilroy. Aging immunity may exacerbate covid-19. *Science*, 369(6501):256–257, 2020.

[28] Fazle E Faisal and Tijana Milenkovic. Dynamic networks reveal key players in aging. *Bioinformatics*, 30(12):1721–1729, 2014.

[29] Davide Bolignano, Francesco Mattace-Raso, Eric JG Sijbrands, and Carmine Zoccali. The aging kidney revisited: a systematic review. *Ageing research reviews*, 14:65–80, 2014.

[30] Csaba Kerepesi, Bálint Daróczy, Ádám Sturm, Tibor Vellai, and András Benczúr. Prediction and characterization of human ageing-related proteins by using machine learning. *Scientific reports*, 8(1):4094, 2018.

- [31] L Abenavoli, C Luigiano, PH Guzzi, N Milic, C Morace, L Stelitano, P Consolo, S Miraglia, S Fagoonee, C Virgilio, et al. Serum adipokine levels in overweight patients and their relationship with non-alcoholic fatty liver disease. *Panminerva medica*, 56(2):189–193, 2014.
- [32] Pietro H Guzzi and Mario Cannataro. -cs: An extension of the tm4 platform to manage affymetrix binary data. *BMC bioinformatics*, 11(1):315, 2010.
- [33] Robi Tacutu, Thomas Craig, Arie Budovsky, Daniel Wuttke, Gilad Lehmann, Dmitri Taranukha, Joana Costa, Vadim E Fraifeld, and Joao Pedro De Magalhaes. Human ageing genomic resources: integrated databases and tools for the biology and genetics of ageing. *Nucleic acids research*, 41(D1):D1027–D1033, 2012.
- [34] Pietro Hiram Guzzi and Tijana Milenkovic. Survey of local and global biological network alignment: the need to reconcile the two sides of the same coin. *Briefings in bioinformatics*, 19(3):472–481, 2018.
- [35] Giovanni Nassa, Roberta Tarallo, Pietro H Guzzi, Lorenzo Ferraro, Francesca Cirillo, Maria Ravo, Ernesto Nola, Marc Baumann, Tuula A Nyman, Mario Cannataro, et al. Comparative analysis of nuclear estrogen receptor alpha and beta interactomes in breast cancer cells. *Molecular BioSystems*, 7(3):667–676, 2011.
- [36] Mikhail V Blagosklonny. From causes of aging to death from covid-19. *Aging (Albany NY)*, 12(11):10004, 2020.
- [37] UpasanaBhattacharyyaandBKThelma. Age-relatedgeneexpressionalterationsbysars-cov-2infectioncontribute to poor prognosis in elderly. *Journal of Genetics*, 99(1):1–9, 2020.
- [38] Kaiwen Jia, Chunmei Cui, Yuanxu Gao, Yuan Zhou, and Qinghua Cui. An analysis of aging-related genes derived from the genotype-tissue expression project (gtex). *Cell death discovery*, 4(1):1–14, 2018.
- [39] Arthur Liberzon, Aravind Subramanian, Reid Pinchback, Helga Thorvaldsdóttir, Pablo Tamayo, and Jill P Mesirov. Molecular signatures database (msigdb) 3.0. *Bioinformatics*, 27(12):1739–1740, 2011.
- [40] Pietro H Guzzi, Daniele Mercatelli, Carmine Ceraolo, and Federico M Giorgi. Master regulator analysis of the sars-cov-2/human interactome. *Journal of clinical medicine*, 9(4):982, 2020.
- [41] David E Gordon, Gwendolyn M Jang, Mehdi Bouhaddou, Jiewei Xu, Kirsten Obernier, Kris M White, Matthew J O’Meara, Veronica V Rezelj, Jeffrey Z Guo, Danielle L Swaney, et al. A sars-cov-2 protein interaction map reveals targets for drug repurposing. *Nature*, pages 1–13, 2020.
- [42] Young-RaeCho, MarcoMina,YanxinLu, NayoungKwon, andPietroHGuzzi. M-finder: Uncoveringfunctionally associated proteins from interactome data integrated with go annotations. *Proteome science*, 11(S1):S3, 2013.
- [43] M Bernabeu-Wittel, JE Ternero-Vega, P Díaz-Jiménez, C Conde-Guzmán, MD Nieto-Martín, L Moreno-Gaviño, J Delgado-Cuesta, M Rincón-Gómez, L Giménez-Miranda, MD Navarro-Amuedo, et al. Death risk

stratification in elderly patients with covid-19. a comparative cohort study in nursing homes outbreaks. Archives of gerontology and geriatrics, 91:104240, 2020.

[44] Damian Szklarczyk, Annika L Gable, Katerina C Nastou, David Lyon, Rebecca Kirsch, Sampo Pyysalo, Nadezhda T Doncheva, Marc Legeay, Tao Fang, Peer Bork, et al. The string database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Research, page gkaa1074, 2020.

[45] David Croft, Gavin O'Kelly, Guanming Wu, Robin Haw, Marc Gillespie, Lisa Matthews, Michael Caudy, Phani Garapati, Gopal Gopinath, Bijay Jassal, et al. Reactome: a database of reactions, pathways and biological processes. Nucleic acids research, 39(suppl_1):D691–D697, 2010.

[46] Paul Shannon, Andrew Markiel, Owen Ozier, Nitin S Baliga, Jonathan T Wang, Daniel Ramage, Nada Amin, Benno Schwikowski, and Trey Ideker. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research, 13(11):2498–2504, 2003.

[47] MR Encarnacion, BM Beata, and D Kenneth. Cause, consequences, and reversal of immune system aging. J Clin Investig, 123:958–65, 2013.

[48] William W Thompson, David K Shay, Eric Weintraub, Lynnette Brammer, Nancy Cox, Larry J Anderson, and Keiji Fukuda. Mortality associated with influenza and respiratory syncytial virus in the united states. Jama, 289(2):179–186, 2003.

[49] Thomas A Reichert, Lone Simonsen, Ashutosh Sharma, Scott A Pardo, David S Fedson, and Mark A Miller. Influenza and the winter increase in mortality in the united states, 1959–1999. American journal of epidemiology, 160(5):492–502, 2004.

[50] Claudio Franceschi and Judith Campisi. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 69(Suppl_1):S4–S9, 2014.

[51] Wenmin Tian, Nan Zhang, Ronghua Jin, Yingmei Feng, Siyuan Wang, Shuaixin Gao, Ruqin Gao, Guizhen Wu, Di Tian, Wenjie Tan, et al. Immune suppression in the early stage of covid-19 disease. Nature communications, 11(1):1–8, 2020.

[52] Massimiliano Bonafè, Francesco Prattichizzo, Angelica Giuliani, Gianluca Storci, Jacopo Sabbatinelli, and Fabiola Olivieri. Inflamm-aging: Why older men are the most susceptible to sars-cov-2 complicated outcomes. Cytokine & Growth Factor Reviews, 2020.

[53] Marco A Marra, Steven JM Jones, Caroline R Astell, Robert A Holt, Angela Brooks-Wilson, Yaron SN Butterfield, Jaswinder Khattra, Jennifer K Asano, Sarah A Barber, Susanna Y Chan, et al. The genome sequence of the sars-associated coronavirus. Science, 300(5624):1399–1404, 2003.

- [54] Riccardo Sgarra, Silvia Pegoraro, Gloria Ros, Carlotta Penzo, Eusebio Chiefari, Daniela Foti, Antonio Brunetti, and Guidalberto Manfioletti. High mobility group a (hmga) proteins: Molecular instigators of breast cancer onset and progression. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1869(2):216–229, 2018.
- [55] Andrea Conte, Simona Paladino, Gaia Bianco, Dominga Fasano, Raffaele Gerlini, Mara Tornincasa, Maurizio Renna, Alfredo Fusco, Donatella Tramontano, and Giovanna Maria Pierantoni. High mobility group a1 protein modulates autophagy in cancer cells. *Cell Death & Differentiation*, 24(11):1948–1962, 2017.
- [56] Payel Sen, Parisha P Shah, Raffaella Nativio, and Shelley L Berger. Epigenetic mechanisms of longevity and aging. *Cell*, 166(4):822–839, 2016.
- [57] Rugang Zhang, Wei Chen, and Peter D Adams. Molecular dissection of formation of senescence-associated heterochromatin foci. *Molecular and cellular biology*, 27(6):2343–2358, 2007.
- [58] Aleksandra S Anisimova, Alexander I Alexandrov, Nadezhda E Makarova, Vadim N Gladyshev, and Sergey E Dmitriev. Protein synthesis and quality control in aging. *Aging (Albany NY)*, 10(12):4269, 2018.
- [59] Brenna C Beckelman, Wenzhong Yang, Nicole P Kasica, Helena R Zimmermann, Xueyan Zhou, C Dirk Keene, Alexey G Ryazanov, Tao Ma, et al. Genetic reduction of eef2 kinase alleviates pathophysiology in alzheimer’s disease model mice. *The Journal of clinical investigation*, 129(2):820–833, 2019.
- [60] Zane A Jaafar and Jeffrey S Kieft. Viral rna structure-based strategies to manipulate translation. *Nature Reviews Microbiology*, 17(2):110–123, 2019.
- [61] David J López, Ander de Blas, Mikel Hurtado, Mikel García-Alija, Jon Mentxaka, Igor de la Arada, María A Urbaneja, Marián Alonso-Mariño, and Sonia Bañuelos. Nucleophosmin interaction with ape1: Insights into dna repair regulation. *DNA repair*, 88:102809, 2020.
- [62] Gianluca Storci, Maria Giulia Bacalini, Francesca Bonifazi, Paolo Garagnani, Sabrina De Carolis, Stefano Salvioli, Fabiola Olivieri, and Massimiliano Bonafè. Ribosomal dna instability: An evolutionary conserved fuel for inflammaging. *Ageing Research Reviews*, 58:101018, 2020.
- [63] C Thiede, E Creutzig, D Reinhardt, G Ehninger, and U Creutzig. Different types of npm1 mutations in children and adults: evidence for an effect of patient age on the prevalence of the tctg-tandem duplication in npm1-exon 12. *Leukemia*, 21(2):366–367, 2007.
- [64] Helena Johansson and Stina Simonsson. Core transcription factors, oct4, sox2 and nanog, individually form complexes with nucleophosmin (npm1) to control embryonic stem (es) cell fate determination. *Aging (Albany NY)*, 2(11):815, 2010.
- [65] Yingchun Zeng, Linbai Ye, Shengli Zhu, Hong Zheng, Peng Zhao, Weijia Cai, Liya Su, Yinglong She, and Zhenghui Wu. The nucleocapsid protein of sars-associated coronavirus inhibits b23 phosphorylation.

Biochemical and biophysical research communications, 369(2):287–291, 2008.

[66] Lei Sun, Pan Li, Xiaohui Ju, Jian Rao, Wenze Huang, Shaojun Zhang, Tuanlin Xiong, Kui Xu, Xiaolin Zhou, Lili Ren, Qiang Ding, Jianwei Wang, and Qiangfeng Cliff Zhang. In vivo structural characterization of the whole sars-cov-2 rna genome identifies host cell target proteins vulnerable to re-purposed drugs. *bioRxiv*, 2020.

[67] Marcel Levi. Covid-19 coagulopathy vs disseminated intravascular coagulation. *Blood advances*, 4(12):2850–2850, 2020.

[68] Myung-Soo Kang and Elliott Kieff. Epstein–barr virus latent genes. *Experimental & molecular medicine*, 47(1):e131–e131, 2015.

[69] Timsy Uppal, Sagarika Banerjee, Zhiguo Sun, Subhash C Verma, and Erle S Robertson. Kshv lta—the master regulator of kshv latency. *Viruses*, 6(12):4961–4998, 2014.

[70] Alerie G de la Fuente, Rayner ML Queiroz, Tanay Ghosh, Christopher E McMurran, Juan F Cubillos, Dwight E Bergles, Denise C Fitzgerald, Clare A Jones, Kathryn S Lilley, Colin P Glover, et al. Changes in the oligodendro-cyte progenitor cell proteome with ageing. *Molecular & Cellular Proteomics*, 2020.

[71] Mehdi Morchikh, Alexandra Cribier, Raoul Raffel, Sonia Amraoui, Julien Cau, Dany Severac, Emeric Dubois, Olivier Schwartz, Yamina Bennasser, and Monsef Benkirane. Hexim1 and neat1 long non-coding rna form a multi-subunit complex that regulates dna-mediated innate immune response. *Molecular cell*, 67(3):387–399, 2017.

[72] Aastha Mishra, Shankar Chanchal, and Mohammad Z Ashraf. Host–viral interactions revealed among shared transcriptomics signatures of ards and thrombosis: A clue into covid-19 pathogenesis. *TH Open: Companion Journal to Thrombosis and Haemostasis*, 4(4):e403, 2020.

[73] Georgia R Kafer and Anthony J Cesare. A survey of essential genome stability genes reveals that replication stress mitigation is critical for peri-implantation embryogenesis. *Frontiers in Cell and Developmental Biology*, 8:416, 2020.

[74] Yong Xiong, Yuan Liu, Liu Cao, Dehe Wang, Ming Guo, Ao Jiang, Dong Guo, Wenjia Hu, Jiayi Yang, Zhidong Tang, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in covid-19 patients. *Emerging microbes & infections*, 9(1):761–770, 2020.

[75] A Rufini, P Tucci, I Celardo, and G Melino. Senescence and aging: the critical roles of p53. *Oncogene*, 32(43):5129–5143, 2013.

Tables

Table 1: P-Values of the enrichment. For each protein we report the significance of the enrichment. A p-value lower than 0.01 means that the interactors are significantly related to ageing. (NS stands for not

significant)

Viral Protein	P-Value	Viral Protein	P-Value
E	NS	S SARS-CoV2 M	6.84E-02
N	NS	NSP1	NS
NSP10	NS	NSP11	0.0001862
NSP12	NS	NSP13	0.002573
NSP14	NS	NSP15	NS
NSP2	0.00182	NSP4	8.32E-03
NSP5	NS	NSP5	NS
NSP6	0.002676	NSP7	NS
NSP8	NS	NSP9	NS
Orf10	NS	Orf3a	5.06E-03
Orf3b	NS	Orf6	NS
Orf7a	0.0001791	Orf8	0.0006913
Orf9b	NS	Orf9c	1.50E-02
Spike	NS		

Table 2: Network Characteristics of M Protein Interactors

number of nodes:	35
number of edges:	61
average node degree:	3.49
avg. local clustering coefficient:	0.422
expected number of edges:	20
PPI enrichment p-value:	1.43e-13

Table 3: Network Characteristics of NSP2 Protein Interactors

number of nodes:	25
number of edges:	46
average node degree:	3.68
avg. local clustering coefficient:	0.566
expected number of edges:	17
PPI enrichment p-value:	3.85e-09

Table 4: Network Characteristics of NSP4 Protein Interactors

number of nodes:	32
number of edges:	45
average node degree:	2.81
avg. local clustering coefficient:	0.45
expected number of edges:	18
PPI enrichment p-value:	7.47e-08

Table 5: Network Characteristics of NSP6 Protein Interactors

number of nodes:	28
number of edges:	40
average node degree:	2.86
avg. local clustering coefficient:	0.535
expected number of edges:	15
PPI enrichment p-value:	4.41e-08

Table 6: Network Characteristics of NSP8 Protein Interactors

number of nodes:	18
number of edges:	30
average node degree:	3.33
avg. local clustering coefficient:	0.565
expected number of edges:	9
PPI enrichment p-value:	1.78e-08

Table 7: Network Characteristics of NSP11 Protein Interactors

number of nodes:	28
number of edges:	44
average node degree:	3.14
avg. local clustering coefficient:	0.505
expected number of edges:	18
PPI enrichment p-value:	2.35e-07

Table 8: Network Characteristics of NSP13 Protein Interactors

number of nodes:	27
number of edges:	58
average node degree:	4.3
avg. local clustering coefficient:	0.582
expected number of edges:	22
PPI enrichment p-value:	1.31e-10

Table 9: Network Characteristics of Orf3a Protein Interactors

number of nodes:	33
number of edges:	87
average node degree:	5.27
avg. local clustering coefficient:	0.631
expected number of edges:	39
PPI enrichment p-value:	3.17e-11

Table 10: Network Characteristics of Orf8 Protein Interactors

number of nodes:	29
number of edges:	40
average node degree:	2.76
avg. local clustering coefficient:	0.465
expected number of edges:	21
PPI enrichment p-value:	0.000115

Table 11: Network Characteristics of Orf9c Protein Interactors

number of nodes:	42
number of edges:	80
average node degree:	3.81
avg. local clustering coefficient:	0.441
expected number of edges:	21
PPI enrichment p-value:	0.000115

Table 12: Network Characteristics

Parameter	Value
number of nodes:	8
number of edges:	9
average node degree:	2.25
avg. local clustering coefficient:	0.667
expected number of edges:	1

Figures

Figure 2

Human PPI Network induced by SARS-CoV-2 Interactors of the M-Protein

Figure 3

Human PPI Network induced by SARS-CoV-2 Interactors of the NSP2 Protein

Figure 4

Human PPI Network induced by SARS-CoV-2 Interactors of the NSP4 Protein

Figure 5

Human PPI Network induced by SARS-CoV-2 Interactors of the NSP6 Protein

Figure 6

Human PPI Network induced by SARS-CoV-2 Interactors of the NSP8 Protein

Figure 7

Human PPI Network induced by SARS-CoV-2 Interactors of the NSP11 Protein

Figure 8

Human PPI Network induced by SARS-CoV-2 Interactors of the NSP13 Protein

Figure 9

Human PPI Network induced by SARS-CoV-2 Interactors of the Orf3a Protein

Figure 10

Human PPI Network induced by SARS-CoV-2 Interactors of the Orf8 Protein

Figure 11

Human PPI Network induced by SARS-CoV-2 Interactors of the Orf9c Protein

Figure 12

Human ageing-related proteins most significantly interacting with SARS-CoV-2 proteins.